Further Observations on the Intestinal Trypanoplasmas of Fishes, with a Note on the Division of Trypanoplasma cyprini in the Crop of a Leech.

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With Plates 9 and 10 and 2 Text-figs.

Contents.

I. General Introduction.

In a previous paper in this journal ('Quart. Journ. Micr. Sci.,' vol. 55, 1910) I have given a fairly detailed account of the morphology and method of division of the normal active form of Trypanoplasma congri.

In attempting to unravel the rest of the life-cycle of this animal, I thought that it might be of some assistance if I could obtain material of the two other Trypanoplasmas which had been described from the gut of fishes, more particularly as up to the present no very detailed account has appeared of these forms.

During the summer of 1910 I was able, while occupying the British Association table at Naples, to obtain a number of Box boops, the stomachs of which were heavily infected with a flagellate which I must regard as identical with the form described by Leger as Trypanoplasma intestinalis.

The most interesting point, however, in connection with my material, is that in life this parasite seemed to me always to possess three anterior flagella, though in stained forms they were not usually easy to unravel. For this reason I have decided to re-name this form Trypanoplasmoides intestinalis. I also found the curious form which Leger was inclined to describe as a female Trypanoplasma, and which Alexeieff has more recently described under the name of Trichomonas Legeri. I am inclined to regard this form, for reasons which I hope to give in a later paper, as the zygote of Trypanoplasmoides intestinalis.

My opportunity of working on the parasite in the stomach of Cyclopterus lumpus arose during a stay at the Scottish Fishery Laboratory at Aberdeen, and I should like to take this opportunity of thanking Dr. Williamson for his ever-ready help in connection with this part of the work.

In this case, again, I am not inclined to regard this parasite as a true Trypanoplasma, and for the reasons given below I have decided to retain for this animal Apstein's name of Heteromita dahlii.

I have not succeeded in finding any very important stages in the life-cycle of Trypanoplasma congri, but I have decided to publish here a short account of some curious rounding-up stages, and also of some abnormal division forms. In a future paper I hope to return to some interesting stages of the parasite in Cyclopterus as well as the forms which I regard as the zygote of Trypanoplasmoides intestinalis.

I should like to take this opportunity of thanking the staff

at the Aquarium at Naples for their kindness and help during my stay there, and I should also like to thank Mr. Elmhirst, the Director of the Marine Biological Station, for the great help he gave me in my work at Millport.

Finally, I should like to thank Miss Robertson for her great kindness in giving me some preparations of the crops of leeches containing dividing Trypanoplasma cyprini, and thus enabling me to form some standard from which it was possible to judge how far these various intestinal flagellates of fishes are removed from the true blood Trypanoplasmas. I should like, also, to thank Professor Minchin for his kindness in allowing me to write up the results of this work in his department at the Lister.

II. Methods.

The methods adopted in this paper were the same as those detailed in my previous account of the division of Trypanoplasma congri, but in addition to the stains mentioned there I used Apathy's hæmatoxylin, followed by his Picro-ammoniak fuchsin.

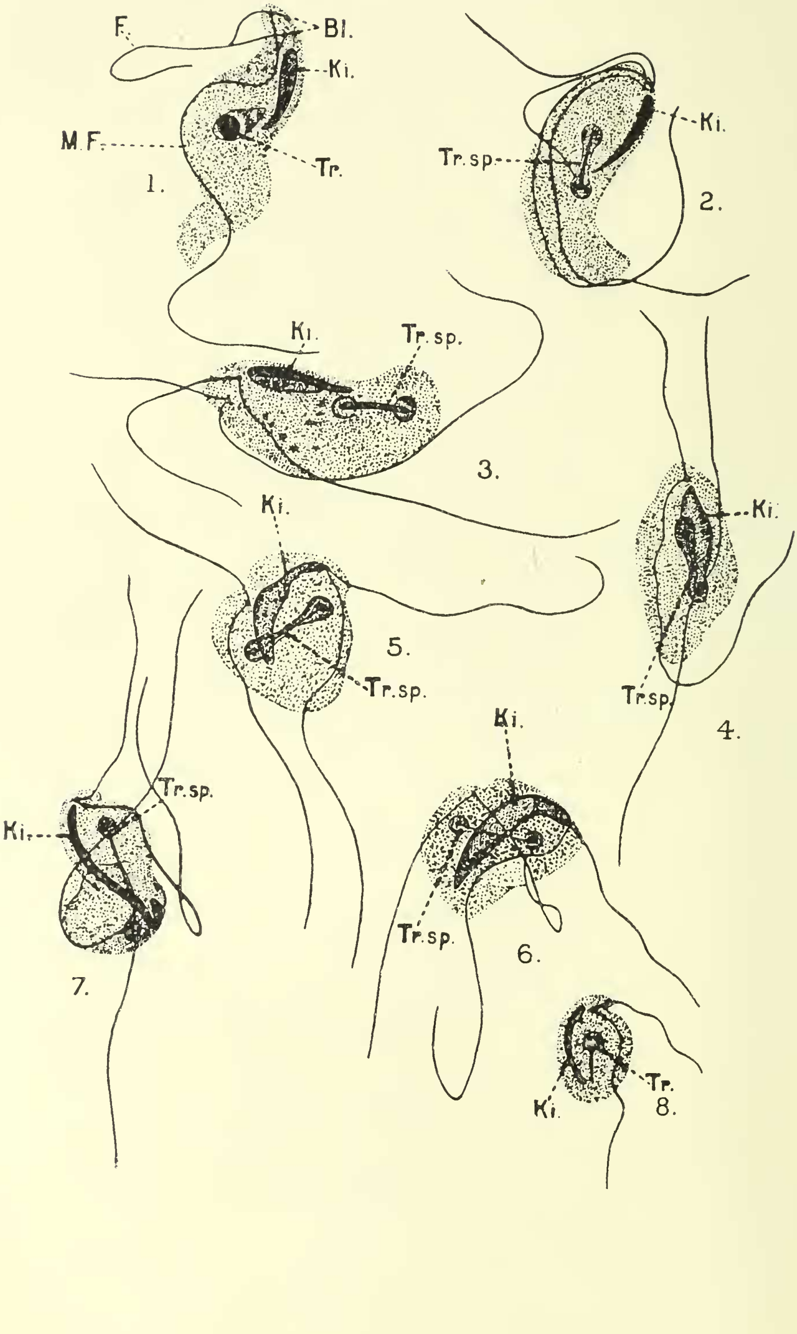
III. Trypanoplasma congri.

I have decided in this part of my paper to give a text-figure of the stages that I have found of the division of Trypanoplasma congri, partly for the sake of comparison with the division stages of the other flagellates from the stomachs of fishes figured below, and partly to elucidate some abnormal division stages which I have found in Trypanoplasma congri. The normal stages of division of Trypanoplasma congri are shown in Text-fig. 1. It will be seen from this that—

(1) The basal granule divides. This is followed immediately by a splitting of the anterior flagellum, and later by the splitting of the posterior flagellum and membrane.

(2) The trophonucleus in the first stage enlarges, the intranuclear chromatin condensing on the karyosome. The trophonucleus assumes first a spindle and later a dumb-bell

TEXT-FIG. 1.


F.
Bl.
Ki.
M.F.
Tr.
1.
Ki.
Tr.sp.
2.
Ki.
Tr.sp.
3.
Ki.
Tr.sp.
4.
Ki.
5.
Tr.sp.
Tr.sp.
Ki.
7.
Ki.
Tr.sp.
6.
Tr.
Ki.
8.

shape, which persists to quite a late stage in division. The karyosome appears to act as an internal division centre, and no trace of individual chromosomes can be seen at any stage of division.

TEXT-FIGURE 1.

Bl. Blepharoplast. F. Free flagellum. Ki. Kinetonucleus. M. F. Membrane flagellum. Tr. Trophonucleus. Tr. sp. Trophonucleus spindle.

Fig. 1.—Early stage of division of Trypanoplasma congri. The whole body of the animal is shorter and stouter. The basal granule has divided, the anterior flagellum is split along about a quarter of its length, and the beginning of the splitting of the posterior flagellum is shown. The kinetonucleus is slightly thicker, and the trophonucleus is distinctly enlarged. The intra-nuclear chromatin granules have probably condensed upon the karyosome, which no longer presents the hard outline characteristic of the resting nucleus.

Fig. 2.—The flagella have now split along their whole length. The karyosome has become drawn out into the characteristic dumb-bell shape within the nuclear membrane.

Fig. 3.—The body of the animal has become still shorter. The kinetonucleus is becoming enlarged and losing its intense capacity for nuclear stain. The dividing trophonucleus is almost parallel to the longitudinal axis of the animal's body.

Fig. 4.—The body of the animal has become still more deformed. The basal granules with their flagella have shifted apart. The kinetonucleus has become thickened, and has now lost its intense capacity for nuclear stains, its lower border is crossed by the trophonuclear dumb-bell.

Fig. 5.—The basal granules with their flagella now lie at opposite sides of the dividing animal. The lower limb of the enlarged kinetonucleus has adopted its characteristic position at right angles to the trophonuclear dumb-bell.

Fig. 6.—A slightly later stage than the previous figure, showing the characteristic relations of the enlarged kinetonucleus and the trophonuclear dumb-bell.

Fig. 7.—A late stage of division. The two products of division are still united by a broad band of cytoplasm, through which the kinetonucleus and trophonuclei still retain their connection.

Fig. 8.—A recently divided form showing the characteristic rounded shape, the elongate kinetonucleus, and the unabsorbed strand which had connected the trophonuclei. The full length of the flagella are not shown.

(3) The kinetonucleus increases in length and divides by a simple transverse constriction.

In some rare cases of division it would appear that part of the cytoplasm, with part of the kinetonucleus, is split off, leaving the trophonucleus spindle in the other individual (Pl. 9, fig. 1). As a result of this very rare form of division forms have been found containing only a kinetonucleus. These, I believe, degenerate. The other individual in this division gives rise, I believe, to rather large forms with two trophonuclei (Pl. 9, fig. 2). I have not been able to discover what the final fate of these forms is.

In addition to the abnormal division forms described above I found some stages of a curious process of rounding-up which seems comparable with that found in some Trypanosomes. In the early stage of this process the Trypanoplasmas assume a characteristic head-to-tail position, the anterior and posterior ends being closely approximated. The membrane flagellum now becomes loosened, at first near its anterior end, and this loosening seems then to travel back from that part to the animal's posterior end. At the same time the approximated body-walls on the inner side disappear, so that in the next stage (Pl. 9, fig. 3) the body of the Trypanoplasma is, roughly, apple-shaped. In this stage the two flagella arise together from a point near the anterior pole, both flagella gradually shorten, and in the still later stages both the flagella have disappeared. The nuclei now begin to undergo a series of changes, the significance of which is by no means clear. The kinetonucleus, which originally showed a bunched, compact appearance, becomes a strand-like structure, and at the same time an elliptical vacuole appears in its neighbourhood. The vacuole increases in size, so that it includes the twisted strands of the kinetonucleus, which at first form a darkly staining axis to the vacuole, but later break up into rod-like masses of chromatin.

The trophonucleus appears to become slightly enlarged in the intermediate stages of this process, but the final form of the Trypanoplasma appears to be a spherical animal with no trace of flagella, and with a large, rounded, vacuolar kinetonucleus and a small trophonucleus. Whether this process may be regarded as the commencement of encystation I am not at present clear.

IV. Heteromita dahlii (Apstein); Trypanoplasma ventricoli (Keysselitz); Diplomastix dahlii (Mobius).

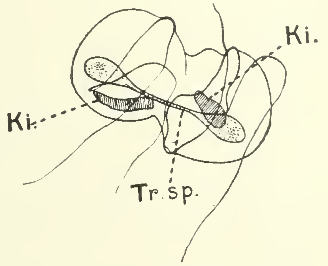
This form appears to have had a remarkably chequered history. It was first discovered by Dahl on March 31st, 1887, and a short account of it was given by Mobius in his "Bruchstucke einer Infusorien Fauna der Kieler Bucht" under the name of Diplomastix dahlii. He described it as a spindle-shaped animal with a flagellum double as long as the body at either pole. The next mention of this flagellate that I can find is in Keysselitz's paper on "Generation und Wirtswechsel von Trypanoplasma Borreli" (p. 37). Keysselitz apparently had overlooked Mobius's work, since he calls the animal Trypanoplasma ventriculi. He gives three figures of the animal under this name, and points out that the blepharoplast is very frequently split into two pieces. Apstein, in his very valuable paper on Cyclopterus lumpus, again gives a short description of this flagellate under the name of Heteromita dahlii, and points out that the name Diplomastix is untenable since the flagella both arise from the anterior pole.

Out of 101 Cyclopterus which Apstein examined he found that 98 were infected. He described the movements of the active forms in some detail, and also some rounded forms which he regards as cysts. He did not succeed in finding any dividing forms, and he draws attention to an interesting mould which is found in association with the flagellates on the stomach-wall. As this animal shows so many points of difference from any other flagellate, free-living or parasitic, of which I have been able to find a description, I have decided to retain for it Apstein's name Heteromita dahlii. In life the animal swims actively, keeping its posterior flagellum in contact with the body-wall, although there is no evidence of a membrane. Along the line of attachment of the posterior flagellum to the body a number of bright granules can be seen according in appearance with those found in Trypanoplasma. At the posterior end of the body a number of large food-vacuoles are to be seen. The animal very often becomes fixed by the posterior end, and well-marked euglenoid movements are shown, both during progression and when stationary. A mouth can be readily made out in life near the base of the anterior flagellum.

In stained preparations the body of Heteromita dahlii is a spindle-shaped structure, slightly elongated at the anterior end, at which a small cytostome can be seen. The two flagella take their origin near the anterior end from two small blepharoplast granules lying anterior to the kinetonucleus. The anterior flagellum is much thickened at its base. The posterior flagellum runs back closely attached to the body of the animal, to end freely, and there is no trace of an undulating membrane. Near the anterior end there is an elongated, darkly staining kinetonucleus; this, as Keysselitz has already pointed out, is very variable in shape, but most frequently it seems to consist of a small, rounded anterior portion connected by a narrow neck with a rod-shaped mass (Pl. 9, fig. 8), but forms are met with in which the kinetonucleus presents quite a different appearance (Pl. 9, fig. 7), and in some cases the process of division of the kinetonucleus is carried still further. The trophonucleus is small and spherical. It contains a large karyosome, but most of the chromatin seems to appear in the form of granules lying between the karyosome and the nuclear membrane. In the earliest stage of division which I have found the body of the animal becomes very rounded (Pl. 9, fig. 8), the flagella have already split along their whole length, and the karyosome within the membrane of the trophonucleus has become elongated. The kinetonucleus is thickened, and shows a slight split at its anterior end. In the next stage one blepharoplast with its attached flagella has travelled back along the animal's body, together with part of the kinetonucleus, which is apparently just breaking through (Pl. 9, fig. 10). In a still later stage (Pl. 9, fig. 11), the animal has become distinctly pear-shaped, and the kinetonuclei lie so that their longitudinal axes are almost at right angles to each other. The trophonucleus spindle during the later stages of division becomes much elongated, and, as is shown in Text-fig. 2, apparently persists right up to the very last stage of division.

As regards the distribution of the parasite, as far as I can discover it is limited in its active form to the stomach. The

Text-fig. 2.



Last stage of division in Heteromita dahlii. Comp. oc. 12 + 1·5 mm. apo. *Ki.* Kinetonucleus. *Tr. sp.* Trophonuclear spindle.

stomachs of the Cyclopterus which I examined give a slightly acid reaction with neutral red. According to Biedermann in Winterstein's 'Handbuch der Vergleichenden Physiologie,' Bd. ii, Jena, 1911, van Herwerden found in Cyclopterus lumpus that "10 c.cm. des filtrierten Magensaftes vom erstgenannten Fische wurden schon neutralisiert von 0·4 c.cm. n/10 NaOH, woraus sich eine Aciditat von nur 0·014 Proz. (als HCl) berechnet."

In order to examine as far as possible the effect on the flagellates of the passage from the stomach into the intestine of its host, I mounted a live smear of the parasite in intestinal juice. The parasites seemed at first to become swollen and revolve round and round instead of exhibiting their usual normal progressive movement. Ten minutes after mounting a number of granular forms were seen, and half-an-hour afterwards nearly all the parasites were motionless.

Apstein, in his paper referred to above, has paid some attention to the physiology of this form. He states on page 11 of this paper: "Handelt es sich in unserem Falle um parasitismus, oder spielen die Flagellaten vielleicht eine wichtige physiologische Rolle? Ich mochte das letzere glauben. Das regelmassige vorkommen wurde allein nicht dafur sprechen Die Wasseraufnahme aber hat vielleicht den Zweck, den Magensaft des Seehasen so zu verdünnen, dass die Flagellaten im Magen leben können, andererseits sie verdaut werden würden Welchen Nutzen die Flagellaten aber fur die Seehasen haben, vermag ich nicht anzugeben."

Under these circumstances I thought it might be of some interest to compare the length of life of the flagellate after the death of its host on a piece of stomach placed in sea-water with that on the stomach-wall of the dead animal. The experiment was not very convincing, however, as the mucus on the inner surface of the stomach-wall seemed to be coagulated to a certain extent on placing it in sea-water; still, forty-eight hours after the death of the host a few active forms were still found in the piece of stomach placed in sea-water, and quite a large number were found on the stomach-wall of the host. In the intestine of the Cyclopterus no trace of the parasite could be found. In the gall-bladder a very small Trichomastix was found, and the rectum was crowded with bacteria and a very large spirochæta.

V.

Trypanoplasmoides intestinalis (Leger); Trypanoplasma intestinalis (Leger); Cryptobia intestinalis (Alexeieff).

This animal was first described by Leger, without illustrations, under the name of Trypanoplasma intestinalis in 1905 ('C.R. Soc. Biol.,' t. 58) Leger described this parasite as a true Trypanoplasma. He also drew attention to the presence of a form with three flagella characterised by very curious movements which he was inclined to regard as a female Trypanoplasma.

During the month of July, in 1910, I had the opportunity during a stay at Naples of examining nine individuals of Box-boops, all of which were heavily infected with this flagellate. I was immediately struck, in the examination of the live individuals, by the fact that all of them when carefully examined showed the presence of three free anterior flagella. After the publication of Alexeieff's note I re-examined my stained preparations and found that it was extraordinarily difficult to show the presence of these flagella on the stained forms. During May of 1912 I examined thirteen Box-boops, and again convinced myself of the existence of these three anterior flagella in living forms of this animal. Upon this occasion I also met with the large amœboid form which Alexeieff has called Trichomonas Legeri. I have found some evidence which I hope to publish in a later note pointing to the fact that this form is really the zygote of Trypanoplasmoides intestinalis. In a more recent paper, "Sur la revision du genre Bodo Ehrbg." ('Arch. fur Protistenkunde,' Bd. xxvi, 1912), Alexeieff has re-named the normal form of this flagellate Cryptobia intestinalis. Taking into consideration the relations of the flagella in this form, I have decided to re-name the animal Trypanoplasmoides intestinalis.

In life Trypanoplasmoides is a roughly carrot-shaped organism with a rather blunt anterior end, from which three free flagella take their origin, while a fourth flagellum turns back along the body in connection with the undulating membrane to end freely at the posterior end. The three anterior free flagella are relatively easily seen in living forms, but in stained forms they are usually very difficult to make out, as they are very fine and are generally twisted together in a single strand. The animals in life move in rather a hesitating manner, in a way that is far more suggestive of a

Trichomastix than of a true Trypanoplasma. As the animal passes forward its anterior end rotates in a small circle from right to left, like the hands of a watch. The number of anterior flagella can best be seen in forms which are anchored by their posterior end; the flagella then strike upwards individually and swing back together. As regards the distribution of the parasites in the gut, it is interesting to note that in the cases I have examined the stomach was heavily infected as low down as the Pylorus. Below this no trace of Trypanoplasmoides could be found. The next portion of the gut, viz. the pyloric cæca and the commencement of the intestine, were, in the case of the fishes I examined, free from all flagellates. The rectum was in most cases seen to be heavily infected with an Octomitus and the interesting ciliate Opalina saturnalis, which has been described by Leger and Duboscq.

In the stained forms the flagella seem to take their origin from a small basal granule lying in front of the darkly staining mass of the kinetonucleus. The kinetonucleus is very variable in shape; generally it is more or less sausage shaped, but it is often drawn out in the middle, so that it may appear as if divided into two or more blocks. Such appearances of the kinetonucleus have often been figured as evidence of division in this and similar forms, but I think it will be clear from the description of the division given below that they have really nothing to do with this process. (Pl. 9, figs. 14, 15).

The trophonucleus is generally rather small and spherical, and contains a fairly large karyosome. Some granules of chromatin are usually found on the membrane of the nucleus. Trypanoplasmoides seem to have a very large range of variation in size, and it is curious to note that the forms undergoing division are generally found amongst either the smaller or the medium sized animals.

The details of the division of Trypanoplasmoides are rather difficult to make out, particularly as regards the behaviour of the flagella. In the early stages of division the blepharoplast divides, and at the same time the undulating membrane splits. One blepharoplast, attached to its portion of the split undulating membrane, and carrying probably two of the free flagella, moves away from its original position. At the same time the anterior end of the kinetonucleus splits, and it would appear from the later stages of division that this part is carried over in connection with the migrating blepharoplast to a position on the opposite side of the animal. The only thing that is abundantly clear in this division of the kinetonucleus is that no trace of a mitotic figure can be found, and it is hard to see what part a mitotic figure could play in such a division. During division the chromatin in the trophonucleus becomes condensed within the nuclear membrane into a dumb-bell-shaped structure. In the later stages the dividing trophonuclei move apart, so that the handle of the dumb-bell becomes much elongated, and still persists at a stage (Pl. 9, fig. 20) at which the cytoplasm at the anterior end is already showing distinct signs of division.

In the case of Trypanoplasmoides I have also found rounded forms, which apparently result in the structure shown in Pl. 9, fig. 21. I have not any evidence to decide whether this is a stage of the process of encystation. I do not propose in this paper entering into a discussion as to the origin and meaning of the curious form which has been named by Alexeieff, Trichomonas legeri, as at present I have no evidence as to its final fate. It would appear, however, from the preparations that I have examined that two Trypanoplasmoides conjugate, and the trophonuclei fuse together. At this point possibly the kinetonuclei break down, although on this point I am rather doubtful. A great part of the chromatin is thrown out into the cytoplasm of the conjugating individuals, and the animal finally comes to possess the curious elongated nucleus with its tiny karyosome characteristic of this form. I am not at all clear as to the behaviour of the flagella during this process, but the undulating membrane seems to be absorbed at a relatively early stage of the process, and I believe this is also true of the free flagella at a slightly later stage. I hope to describe this process in greater detail in a forthcoming paper.

VI. The Division of Trypanoplasma cyprini in the Crop of the Leech.

For the purpose of examining how far the various parasites described above approximate to the type of the true trypanoplasmas in the blood of fishes, it is obvious that they must be compared with the latter in the various stages of their life-cycle. Unfortunately, not much appears to be known of the morphology of trypanoplasmas. Keysselitz, in his paper, "Generations- und Wirtswechsel von Trypanoplasma borreli, Laveran et Mesnil," published a long account of the life-cycle of a blood Trypanoplasma, but his figures of division, owing to the unfortunate adoption of a dry technique, leave much to be desired. This point has already been insisted on by Minchin in his paper, "Some Observations on the Flagellates Parasitic in the Blood of Freshwater Fishes."

Owing to the kindness of Miss Robertson, I recently had the opportunity of examining some division stages of Trypanoplasma cyprini in the crop of a leech. Miss Robertson has shown in her paper on "The Transmission of Flagellates living in the Blood of Certain Fresh-water Fishes" ('Phil. Trans.,' B, vol. ccii.), that—"When first taken into the crop of the leech, the Trypanoplasmas become rather broader and bulkier in appearance, and show a very characteristic flowing kind of motion. After the first few hours they are frequently to be seen in division; the last stage of this process is passed through with great rapidity. On the second day after feeding, slender forms, somewhat comma-shaped, appear in small numbers; they are much less undulating in their movements than the broader forms. These two types persist side by side for some time, the slender forms gradually increasing in number; intermediate forms are also present. After some days the slender forms move forward into the proboscis-sheath. They may occupy this situation as early as the sixth day after feeding. As time goes on they come to lie there in incredible numbers, and very generally attach themselves to the wall by their anterior end, i. e. the end with the free flagellum." It is obvious that in the case of these preparations we are dealing with a specialised type of division resulting in the production of a more elongated trypanoplasma than the normal blood type (Miss Robertson's so-called "comma" type), which is destined to develop into the elongate proboscis form, the true infective form.

The form of Trypanoplasma which is apparently undergoing division in these preparations is shown in Pl. 10, fig. 22, and the elongated infective stage in Pl. 10, fig. 23.

Unfortunately, in the preparations I have examined the early stages of division are very rare, though the later stages are exceedingly abundant. As far as I have been able to see, the new flagella arise by splitting, but this is at present a somewhat doubtful point. Whatever the origin of the new flagella may be, it is obvious, from Pl. 10, figs. 24 and 25, that one blepharoplast after division travels backwards as the kinetonucleus elongates. The elongated kinetonucleus then divides by means of a simple transverse constriction. The division of the trophonucleus is precisely similar to that which has been previously described for Trypanoplasma congri, and it is interesting to note that here again, as is shown in Pl. 10, fig. 29, the products of division may show for a short time the remains of the strand connecting the dividing trophonuclei.

VII. Conclusions.

It would appear that at present the nomenclature of the bimastigate flagellates is in a state of almost inextricable confusion. This state appears to me to depend largely on two factors—(1) the discovery of a free-living bimastigate form with a kinetonucleus (Prowazekia), and the consequent doubt as to how many of the original species of the genus Bodo may not turn out to be Prowazekia; (2) the recent attempt made by certain authors, on the assumption that the parasite in the receptaculum seminis of snails is identical with the true Trypanoplasmas, to place the intestinal Trypanoplasmas in the genus Cryptobia.

It appears to me that the first step in surmounting these difficulties must be made by a careful study of the genus Cryptobia, the parasite of the snail.

In Friedrich's original paper clear division stages were shown, and these were all of a type which seems very different from that found in true Trypanoplasmas. It does not seem that the more recent account of the division of this form by Jollos can be regarded as replacing Friedrich's original account, since it seems to me more than probable that many of the stages figured by Jollos do not represent division at all, and in any case his series is very incomplete.

On the whole, it seems safest at present to assume that Cryptobia is not a true Trypanoplasma, though the only distinctive feature appears at present to be the absence of distinct undulating membrane in Cryptobia. It seems probable, however, from Friedrich's paper that other distinguishing features would be found by a careful study of the other stages of this animal.

In this connection it is important to remember that Max Kuhn, who has recently studied these parasites in a very large number of snails, has also expressed some doubts as to how far they can be regarded as true Trypanoplasmas ('Vid Die Trypanoplasmen und deren Verbreitung in einheimische und auslandischen Schnecken,' p. 70):

"Die letztere die den Randfaden einer Undulirende Membran darstellt ist sehr viel lockerer mit dem Korper verbunden wie bei dem Trypanosomen. So dass sich haufig auf gefarbter Praparaten die Randgeissel mehr oder wenig frei zeigt und somanchmal scheint als ob hier ein Vertreter der Gattung Prowazekia vorliegt."

If this view that Cryptobia differs from the true Trypanoplasma be accepted, there now remains for discussion the question as to which, if any, of the so-called intestinal Trypanoplasmas should be included in the genus Trypanoplasma.

If the figures of the dividing Trypanoplasma cyprini shown in Pl. 10 are compared with the figures of the division of Trypanoplasma congri shown in Text-fig. 1, bearing in mind that the division in the case of Trypanoplasma cyprini is a specialised one resulting in the production of a slender form, it will be obvious that there are many points of similarity. I think that these are probably sufficient to retain provisionally the Conger parasite in the genus Trypanoplasma. The parasite in Cyclopterus, *Heteromita dahlii*, appears to me to be separated from the true Trypanoplasma by many important points of difference. Firstly, the absence of an undulating membrane; secondly, the presence of a mouth and ingestion of food; and thirdly, its method of division. I believe that a more detailed examination of this parasite will lead to further points of difference being discovered, particularly in connection with the process of encystation, but these I must leave for a later paper.

As regards the parasites in the stomach of Box, if my view as to the presence of three free flagella in this form be accepted, it is obvious that we are dealing here with a form which is much more closely allied to Trichomonas than to Trypanoplasma. In this case it will be obvious that the kinetonucleus in Trypanoplasmoides is probably not homologous with the similar structures in the other forms. Apart from the structure of the flagella, there are many other points of difference between Trypanoplasmoides and the true Trypanoplasma, viz. the presence of a distinct cytostome in Trypanoplasmoides, the method of movement, and the method of division.

I should now like to refer shortly to a structure to which it seems to me far too much importance has been attached in recent years—the kinetonucleus. If Jollos's description of the division of this structure were to be accepted this importance would be justified, but I think that from the descriptions of the division of the flagellates given above it will be admitted that the evidence for Jollos's view of the division of the kinetonucleus by means of a mitotic spindle is by no means conclusive. I believe that as a definition of a kinetonucleus all we are at present justified in stating is that the kinetonucleus is a mass, staining darkly with chromatin stains, found near the base of the flagella in some flagellates. That this mass is necessarily homologous in all flagellates appears to me a doubtful assumption, and I believe that when the intestinal flagellates and the free-living Bodos are more closely examined we may find the kinetonucleus in forms for which it would be very difficult to establish a homology.

Finally, it appears to me that the too ready acceptance of the view that the kinetonucleus is a true nucleus comparable to the trophonucleus has led numerous protozoologists of late years into a region of unjustifiable and unprofitable hypothesis.

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EXPLANATION OF PLATES 9 AND 10.

Illustrating Mr. C. H. Martin's paper, 'Further Observations on the Intestinal Trypanoplasmas of Fishes, with a Note on the Division of Trypanoplasma cyprini in the Crop of a Leech.'

Lettering.

Bl. Blepharoplast. *F.* Free flagellum. *F.F.* Free flagella. *Ki.* Kinetonucleus. *M.F.* Membrane flagellum. *P.F.* Posterior trailing flagellum. *Tr.* Trophonucleus. *Tr. sp.* Trophonucleus spindle.

PLATE 9.

[Figs. 1–6 and 14–21 inclusive were drawn under Zeiss comp. oc. 18 and 1·5 mm. apochromat. Figs. 7–13 were drawn under Zeiss comp. oc. 18 and 2 mm. apochromat.]

Fig. 1.—Trypanoplasma congri. Abnormal division product containing the entire trophonuclear spindle.

Fig. 2.—Trypanoplasma congri. Abnormal individual with two trophonuclei.

Fig. 3.—Early rounding-up stage of Trypanoplasma congri.

Fig. 4.—Later stage of same process.

Fig. 5.—Still later stage.

Fig. 6.—Final stage of rounding-up in Trypanoplasma congri.

Fig. 7.—Normal active individual Heteromita dahlii.

Fig. 8.—Stage preparatory to division in Heteromita dahlii.

Fig. 9.—Early stage of division in Heteromita dahlii.

Fig. 10.—Later stage of division; one blepharoplast with its attached flagella has travelled back and the division of the kinetonucleus is almost complete.

Fig. 11.—The body of the dividing individual has become pear-shaped and the dividing kinetonuclei are now at angles to each other.

Fig. 12.—Later stage of division showing elongation of the dividing trophonucleus.

Fig. 13.—Later stage of division in Heteromita dahlii.

Fig. 14.—Normal active individual of Trypanoplasmoides intestinalis showing three anterior flagella twined together.

Fig. 15.—Active individual of Trypanoplasmoides intestinalis showing origin of three free anterior flagella. In this specimen the flagellum of the undulating membrane is broken away from the body.

Fig. 16.—Early stage of division in Trypanoplasmoides intestinalis—the blepharoplasts have divided. In this specimen two free flagella were attached to each blepharoplast.

Fig. 17.—Slightly later stage of division. The second blepharoplast is not shown.

Fig. 18.—Later stage of division.

Fig. 19.—Later stage of division. The kinetonuclei are completely separated.

Fig. 20.—Very late stage of division. The trophonuclei are still connected by a fine strand, though the commencement of the cytoplasmic division is shown at the anterior end.

Fig. 21.—Rounded form of Trypanoplasmoides intestinalis.

PLATE 10.

Fig. 22.—Trypanoplasma cyprini. From the crop of a leech.

Fig. 23.—Trypanoplasma cyprini. Elongate Proboscis type, from the crop of a leech.

Fig. 24.—Trypanoplasma cyprini. Early stage of division, showing the division of the blepharoplasts.

Fig. 25.—Early stage of division, showing the division of the kinetonucleus.

Fig. 26.—Slightly later stage.

Fig. 27.—Later stage of division, the band connecting the dividing trophonuclei still present.

Fig. 28.—Late stages of division.

Fig. 29.—Last stage of division of Trypanoplasma cyprini.